Supporting Information

Calcium Carbonate nanoparticles modulate local pH and inhibit tumor growth *in vivo*

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Supplementary Figures and Captions

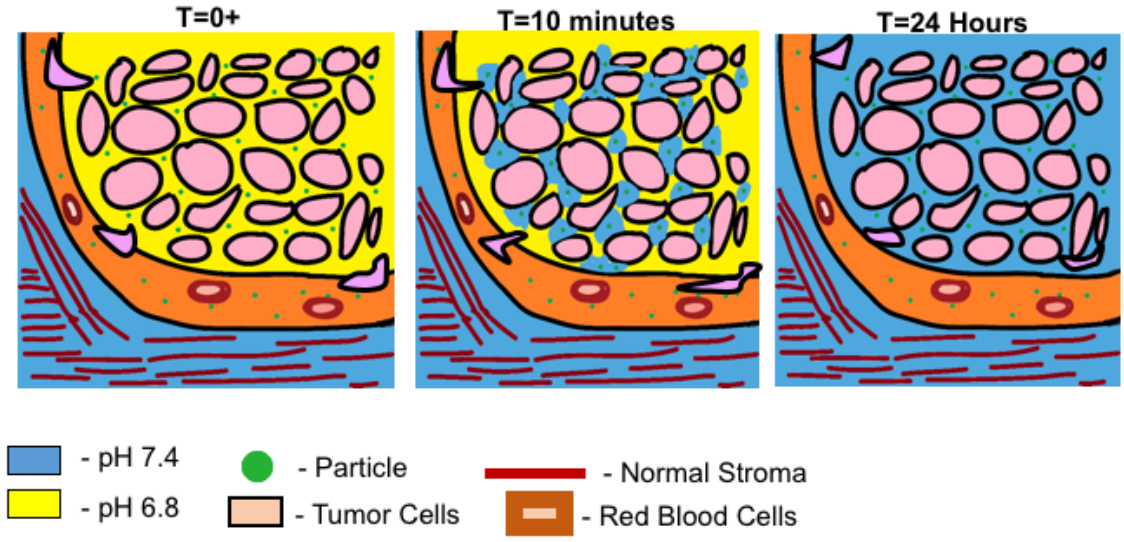

Figure S1. Schema of nano-$CaCO_3$. (a) High dose $CaCO_3$ enters via EPR and increases pH to 7.4. Over time this continuous dose allows chronic maintenance of pH 7.4

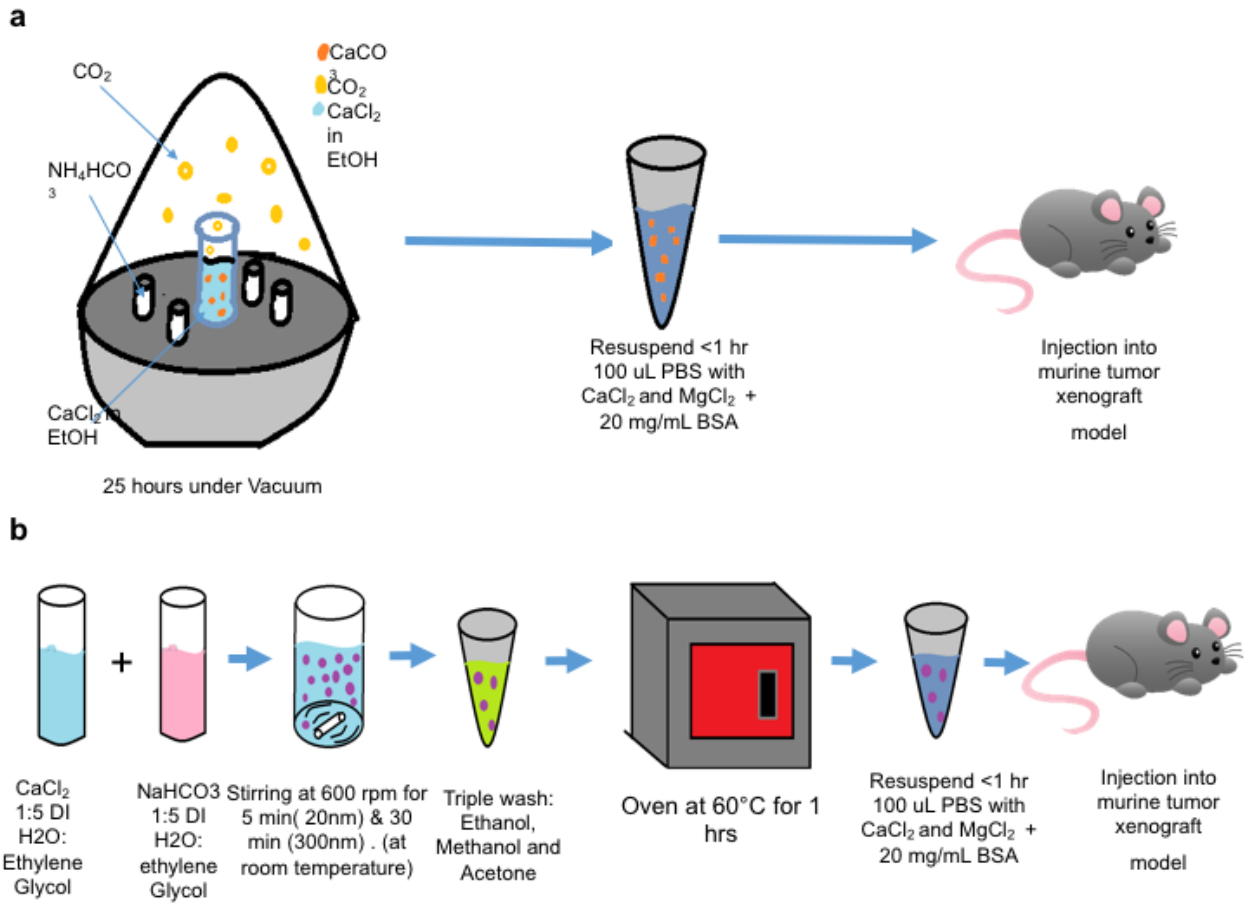

Figure S2. Synthesis and Experimental Schema for nano-$CaCO_3$ synthesis. (a) Synthesis schema for the gas diffusion method for nano-$CaCO_3$ synthesis. (b) Synthesis schema for synthesis of nano-$CaCO_3$ using sol-gel. Both methods can be used to make 20 nm, 100 nm, and 300 nm particles. Synthesis was followed by suspension in an aqueous solvent followed by in vivo experiments in murine models.

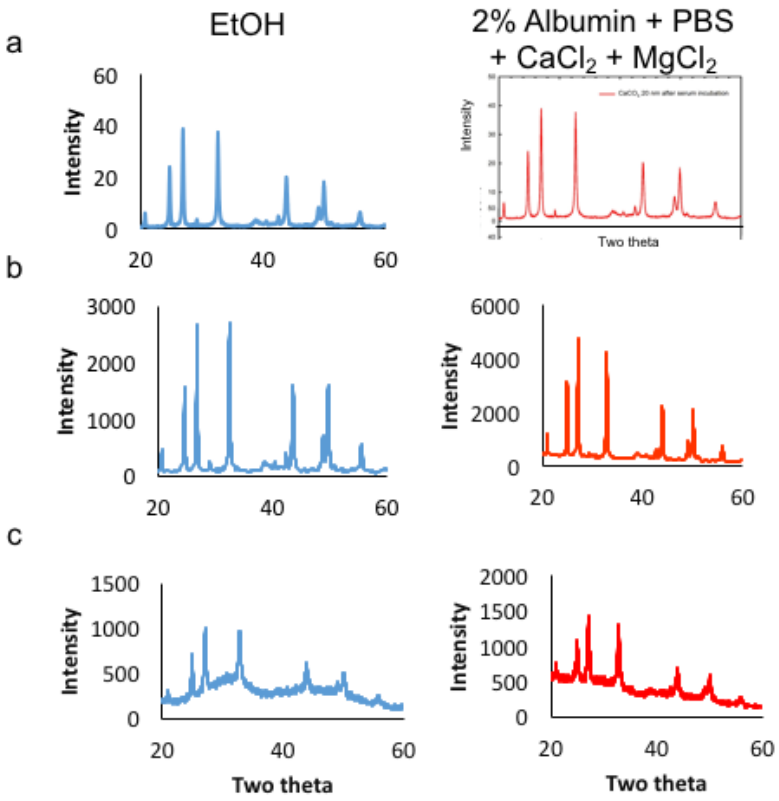

Figure S3. X-Ray diffraction post addition of albumin based solvent shows stability in all three sizes of particles. (a) XRD of 20 nm nano-$CaCO_3$ is identical in EtOH and in 2% albumin + PBS + $CaCl_2$ + $MgCl_2$ solution. (b) XRD of 100 nm nano-$CaCO_3$ is identical in EtOH and in 2% albumin + PBS + $CaCl_2$ + $MgCl_2$ solution. (c) XRD of 200 nm nano-$CaCO_3$ is identical in EtOH and in 2% albumin + PBS + $CaCl_2$ + $MgCl_2$ solution. All plots, when compared to literature, match the structure of vaterite.

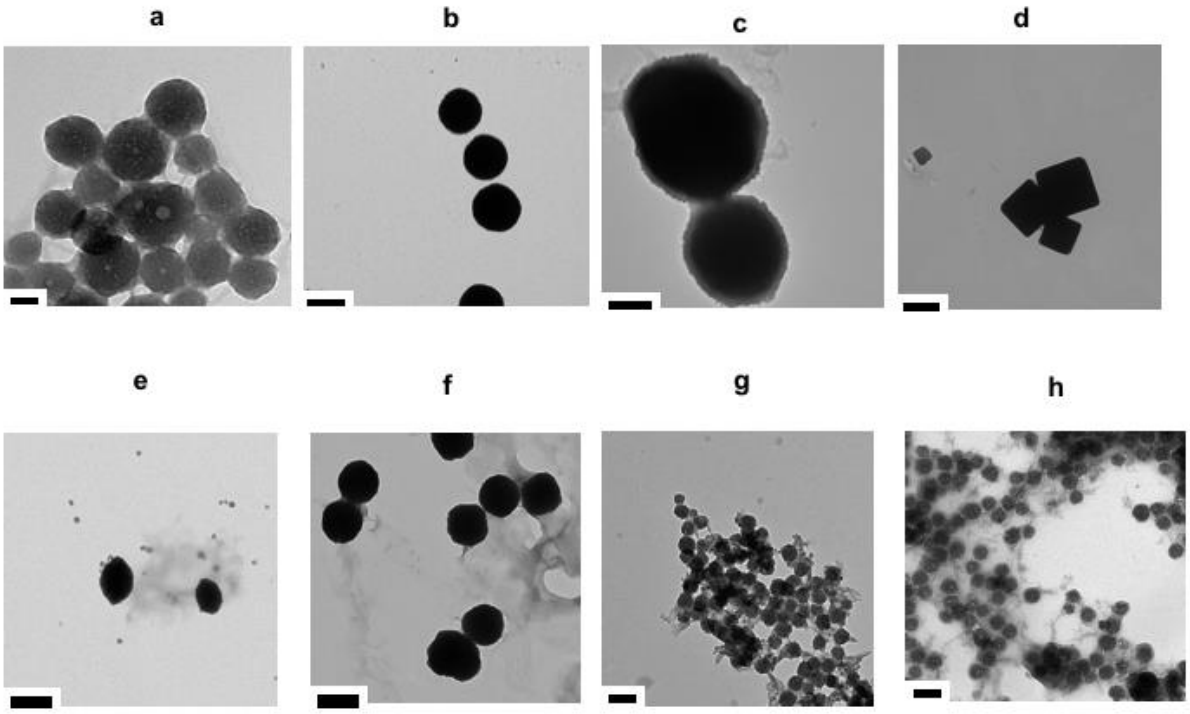

Figure S4. $CaCO_3$ grows in size in a variety of solvent incubations for 100 nm nano-$CaCO_3$ except albumin containing solutions. (a), At synthesis, particles are monodisperse and 100 nm in size. Scale bar represents 50 nm. (b), When placed into deionized H_2O, particles rapidly grow to be > 1 μm. Scale bar is 1 μm. (c), When 100 nm nano-$CaCO_3$ is placed into 15 mM saline, particles grow to greater the > 1 μm. Scale is 1 μm. (d), When placed into PBS, particles become cuboidal in shape and large. Scale bar is 1 μm. (e), Coating particles with a hydrophobic coating using Poly-Vinyl Pyrrolidone (PVP), slows reaction and makes growth slightly slow. Scale bar is 0.5 μm. (f), Coating particles with a hydrophobic coating- using Poly-2-Vinyl Pyrrolidone (PVP), slows reaction and makes growth slightly slow. Scale bar is 0.5 μm. (g), Adding $CaCl_2$ and $MgCl_2$ to the PBS solution slows $CaCO_3$ growth, with some reaction appearing to occur. Scale is 200 nm. (h), Adding 2% albumin to a solvent of PBS + $CaCl_2$ + $MgCl_2$ preserves particle morphology.

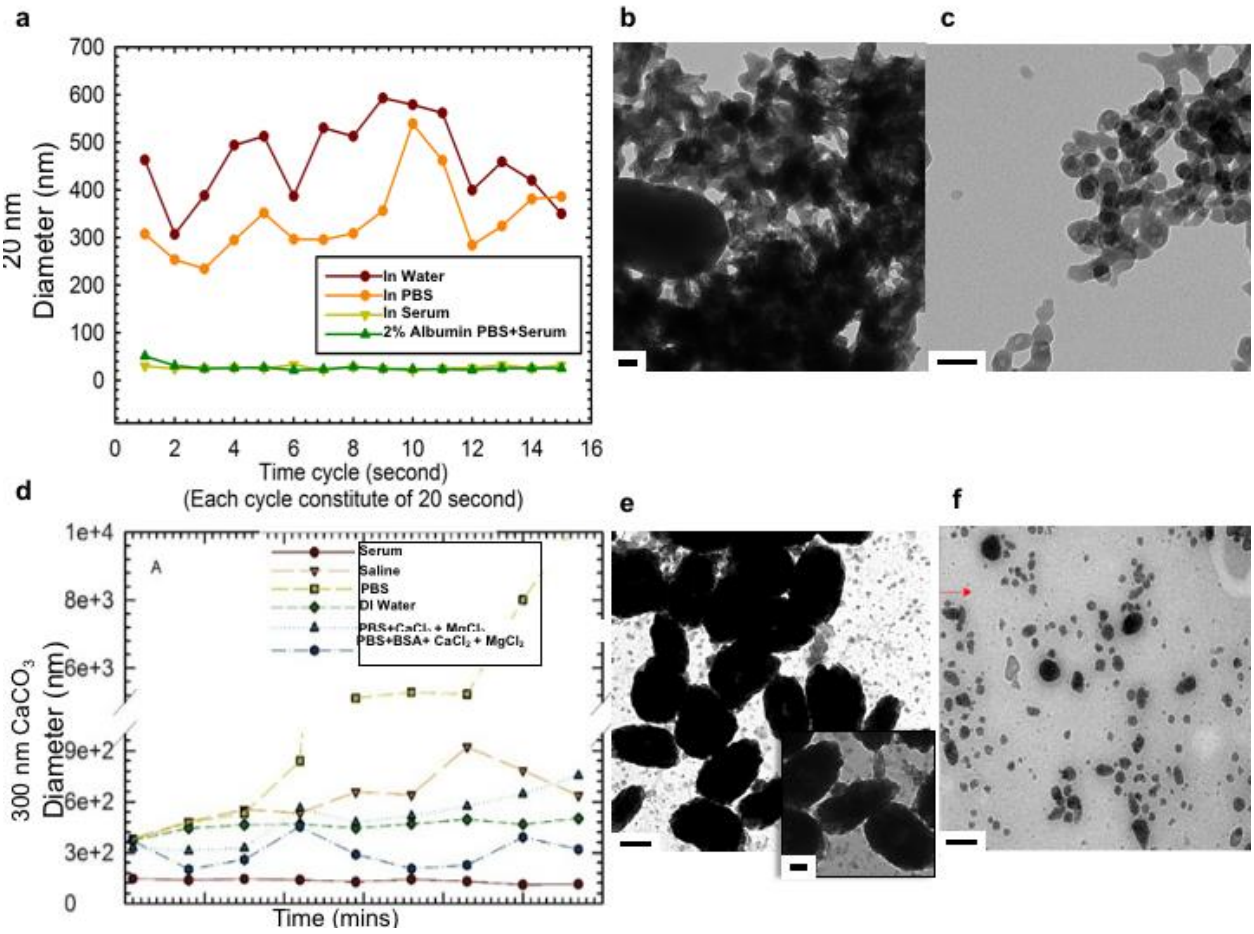

Figure S5. 20 nm and 300 nm particles show stability in albumin containing solution and in serum. (a) DLS results over time in a variety of aqueous solvents for 20 nm particles demonstrates that particles on addition to serum separate from an aggregate in 2% albumin. (b), TEM of 20 nm particles in PBS + $CaCl_2$ + $MgCl_2$ + 2% albumin shows separation . Scale bar is 200 nm. (c), TEM of 20 nm particles post serum incubation shows retention of morphology. (d), DLS results over time in a variety of aqueous solvents for 300 nm particles show the same trend as 20 nm and 100 nm particles in solvent stability. (e), 300 nm particles under 2% albumin + PBS show some slight increase in size. Scale bar for large image is 200 nm. Scale bar for magnified image is 100 nm. (f), 300 nm particles in serum show retention of shape and morphology. Scale bar is 200 nm.

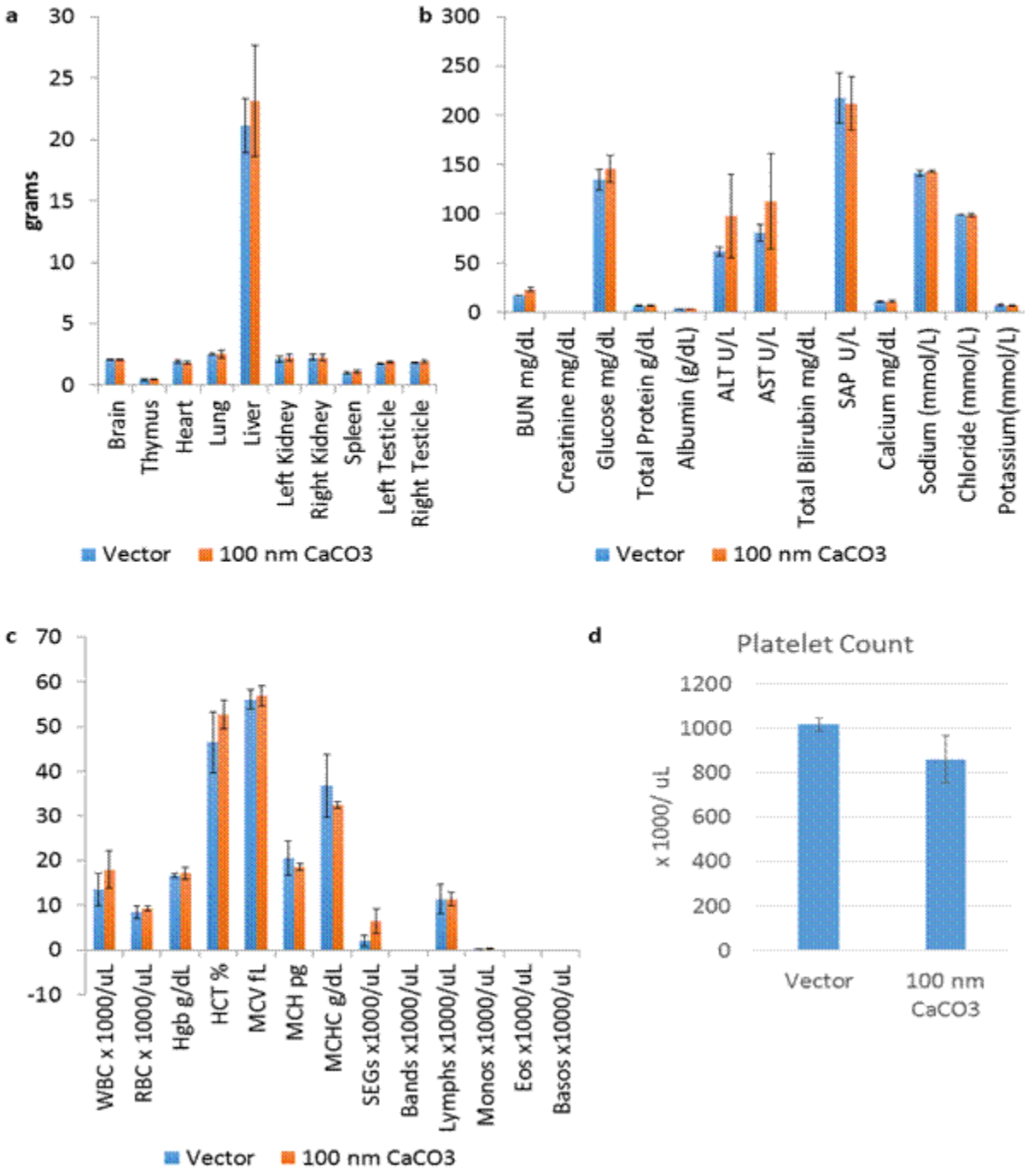

Figure S6. Toxicity study in rats using allometrically dosed CaCO3. (a) Organ weights from rats after 24 hours CaCO3 treatment. (b) Chemistry results of blood metabolites after 24 hours, * represents p< .05. (c) Complete blood count of cells 24 hours after $CaCO_3$ injection. (d) Platelet count 24 hours after CaCO3 inject.